Hepatocellular carcinoma pathogenesis and diagnostic Novel biomarkers: A pathological systemic review

Abhishek Soni[1], **Madhav Nilakanth Mugale**[2]✉

Division of Cancer Biology and Toxicology and Experimental Medicine and Division of Medicinal and Process Chemistry, CSIR-Central Drug Research Institute (CSIR-CDRI), Lucknow, India – 226 031
Senior Scientist, CSIR-Central Drug Research Institute (CSIR-CDRI), Lucknow, India – 226 031

✉**Corresponding author:**
Madhav Nilakanth Mugale: Senior Scientist, CSIR-Central Drug Research Institute (CSIR-CDRI), Lucknow, India – 226 031, Email: madhav.mugale@gmail.com; Fax No.- +91-522-2771941/ Ext. No.4587
ORCID no. HTTP: //orcid.org/ 0000-0003-3138-3860

Article History
Received: 26 March 2020
Accepted: 01 May 2020
Published: May 2020

Citation
Abhishek Soni, Madhav Nilakanth Mugale. Hepatocellular carcinoma pathogenesis and diagnostic Novel biomarkers: A pathological systemic review. *Drug Discovery*, 2020, 14(33), 119-129

ISSN 2278–540X; EISSN 2278–5396

General Note
Article is recommended to print as color version in recycled paper. *Save Trees, Save Climate*.

ABSTRACT

Hepatocellular carcinoma (HCC) is the most frequent and predominant malignant tumor of the liver having a grave prognosis, and regularly diagnosed at an advanced stage. HCC is a multistep process involving the progressive accumulation of molecular variations signifying distinct molecular and cellular events. In a study involving the liver, it has been found that chronic liver diseases such as hepatitis B and hepatitis C amenable for HCC. Other risk factors for growing HCC includes non-alcoholic fatty liver disease (NAFLD) non-alcoholic steatohepatitis (NASH), chronic consumption of aflatoxin-contaminated food, iron overload, and the presence of a range of genetic metabolic diseases. For diagnosis of disease status, a number of various biomarkers such as Alfa-fetoprotein, GPC-3, GP-73, squamous cell carcinoma antigen, des-γ-carboxy prothrombin, circulating miRNAs and cancer stem cell marker is used to diagnosed and treat HCC. Biomarkers signify an enormously convenient way to detect disease progression and accurate analysis. This review aims to broaden our modern notion of the most relevant pathogenesis and molecular pathways involved in the development, progression and recent novel biomarkers involved in HCC.

Keywords: AFP; Hepatocellular carcinoma; HBV; HCV; MiRNA

1. INTRODUCTION

Liver is commonly affecting organ of the body globally. Approximately 7.5 lakh of new cases of HCC and liver disorder per year globally found.(1) In today's scenario HCC (hepatocellular carcinoma) is a predominantly liver disease usually found in developed countries. It ranks 3rd in the world among cancer-related deaths.(2) Many cases of HCC are diagnosed at an early stage and increase in incidence rate from 27% between 1992 and 1999 to 44% between 2006 and 2012 (63% relative increase). The reason behind its improvement is due to better diagnosis and proper documentation of the liver abnormally HCC stage.(3) Patient survival with HCC has only been marginally improved over the last two decades. Between 1981-98, the 5-year survival rate only improves from 2% to 5%. The reason for the poor survival rate is the late diagnose of HCC.(4) Development of hepatocarcinogenesis occurs mainly due to cirrhosis, chronic hepatitis B & C virus infection, non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) and cryptogenic cirrhosis.(2, 5) Other causes include aflatoxin exposure, iron overload, alcohol consumption,(6) primary biliary cirrhosis, and the presence of various genetic metabolic diseases like hereditary hemochromatosis, tyrosinemia, and alfa1-antitrypsin deficiency.(7) Hepatocellular carcinoma is an end result of many liver disorders commonly allied with HBV and HCV.(8) In 2012, analysis was done in 170000 new cancer cases and found that HCV accounted for 7.8% of cases.(9) The extension of HCV-related HCC change with both geographic location and ethnicity. Most cases of HCC related to HCV are seen in South America, Japan, Europe whereas HBV is seen in Asia and Africa. (9) Current statistics gathered by European association for the study of the liver, Asian pacific association, American Association and national comprehensive cancer network recommend HCC surveillance every 6 months. Chronic HCC leads to cirrhosis which is present 90% of patients with HCC.(3) The diagnosis of HCC is achieved by using specific biomarkers for prognosis of the pathological stage of HCC.(10) Biomarkers can be differentiated into the following categories based on their uses like 1 predictive biomarkers, predict response to specific therapeutic interventions. 2 A prognostic biomarker is used to diagnose disease recurrence and progression in the future. 3 A diagnostic biomarker is used to detect metastasis and status of disease conditions in a particular organ of the body.(11) The need for selective biomarkers is due to the fact that HCC cells express various factors like platelet derived growth factor (PDGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) etc. Leads to uncontrolled cellular proliferation. This uncontrolled cellular proliferation makes it difficult to analyse pathological state of HCC.(12, 13) Alpha-fetoprotein(AFP) is widely used as a serum biomarker but in many cases of HCC patient do not have elevated serum AFP level, it's also reported 31.3 % of Non-AFP tumour in cohort study of 665 patient. Therefore identification of specific biomarker is necessary to achieve accurate stage of diagnosis of liver cancer.(14).

Types of liver cancer

Liver cancer is classified on the type of cells involved

1. HCC is shown by approximate 75% of all hepatocellular cells. The most common cause of HCC is liver cirrhosis occurring due to the alcohol or infection with hepatitis B or C.
2. Fibrolamellar HCC is a rare type of HCC that comprises 1–9% of all HCCs. It occurs in adolescents and young adults without underlying liver disease.
3. Bile duct cancer scientifically known as cholangiocarcinoma occurs in ducts carrying bile juice from the liver to the gallbladder. The cholangiocarcinoma is found in 10-20% of cases of liver cancer.
4. Angiosarcoma that is also known as hemangiosarcoma, is seen in about 1% of all liver cancer cases. Angiosarcoma originates from blood vessels of the liver and it grows quickly and usually diagnosed during advanced stages.
5. Secondary liver cancer is metastasis into distant organs. Sometimes cells break away from the primary cancer and are carried in the bloodstream to part of the liver.(15)

2. AETIOLOGY OF MOLECULAR PATHOGENESIS OF HCC

Cirrhosis

Cirrhosis is the result of most chronic liver diseases leads to HCC.(16) and this reduces the ability of hepatocyte proliferation that indicates that the regenerative capacity of the liver is decreased as well as the start nodule formation and alteration in blood flow which causes early development of HCC.(17) And at the same time increased levels of cytokines are responsible for hepatic inflammation, cholestasis and ultimately fibrosis. Tumour necrosis factor – Alfa (TNF-α) is a type of cytokine commonly found in all liver diseases. (18) HCV and HBV have been thought to be the main cause of cirrhosis. There are some statistics directly show that 50% of the cases are related to HBV while 25% to HCV.(19) However, it has been observed that in limited patients HBV infection directly increases in HCC without cirrhosis or fibrosis whereas in 85% of patients, HCC is usually developed by cirrhosis.(20)

In the cirrhotic liver condition, the telomere dysfunction and induce alteration in cellular micro and macro environment to enhance cellular proliferation. Telomere dysfunction determines chromosomal instability and reduces the regenerative capacity of the liver with reduced hepatocyte regeneration.(21) It has been proven that telomeres become smaller in the case of cirrhotic liver than in healthy liver.(22) Telomere dysfunction and p53 mutation rapidly develop tumors.(23) Continuously activated oncogenic pathways involved in HCC such as wnt/β- catenin, PI3K/AKT/mTOR, MAPK signalling pathway, etc.(17).

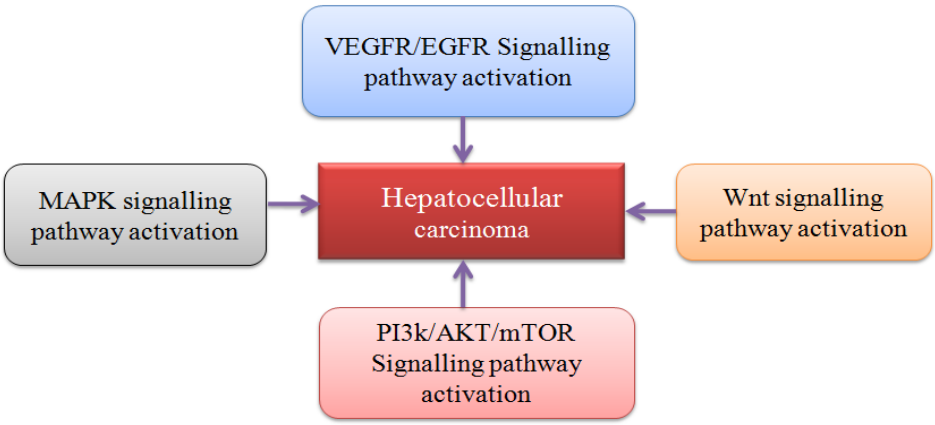


Fig.1 Various pathways involved in liver cancer

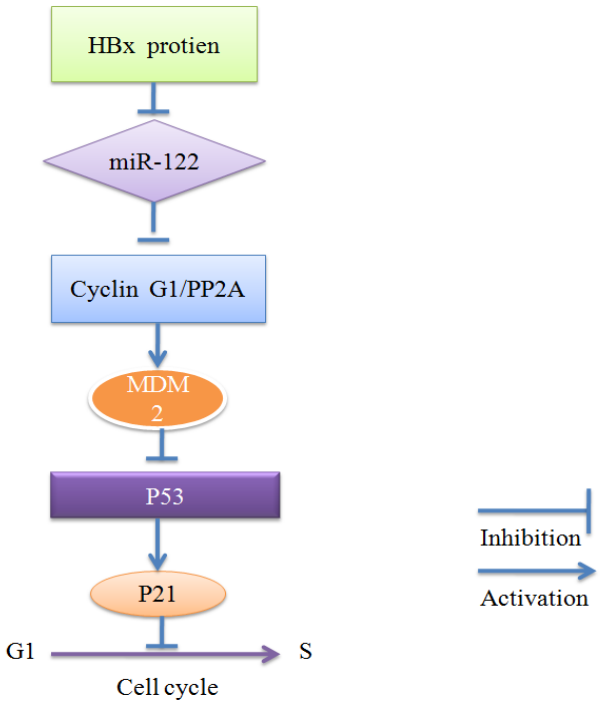
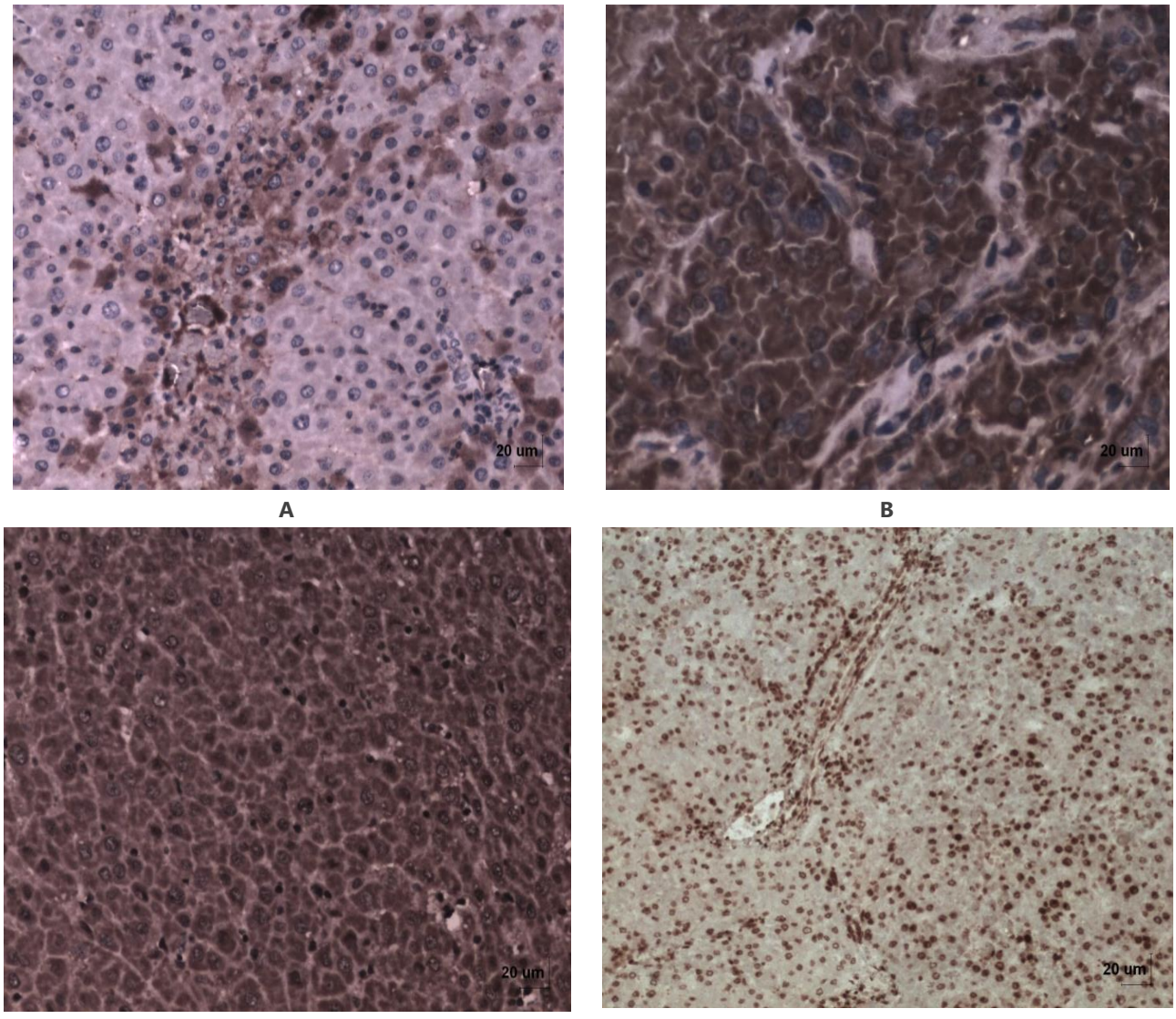


Fig.2 HBx mediated miR-122 suppression which initiates cell proliferation through suppression of p53 mediated activity.

Table 1 showing up and down regulation of miRNAs

Name of MicroRNAs	Expression at HCC tissue	Mechanism
Mir-210	upregulated	metastasis, angiogenesis
Mir-10b	upregulated	migration, invasion
Mir-25	upregulated	emt, apoptosis

Mir-32	upregulated	prognostic marker
Mir-92a	upregulated	cell growth, prognostic marker
Mir-96-5p	upregulated	apoptosis
Mir-107	upregulated	proliferation, prognostic marker
Mir-181a	upregulated	autophagy
Mir-7	downregulated	autophagy, drug resistance
Mir-26	downregulated	autophagy
Mir-29a	downregulated	proliferation, migration
Mir-30a-5p	downregulated	cell growth, apoptosis
Mir-30e	downregulated	emt
Mir-31	downregulated	drug resistance
Mir-31-5p	downregulated	proliferation, migration invasion
Mir-33a	downregulated	prognostic marker



Figs. 3 Rat - Liver – Diethyl nitrosamine induced hepatocarcinogenesis –IHC
Expression of various biomarkers in HCC (A) Alpha-fetoprotein (B) BAX (C) BCL-2 (D) Ki-67 in DEN induce experimental rat model

HBV infection

HBV infection has been considered a significant factor in liver related problems. it promotes several complex pathogenic factors in the liver that play an important role in the development of HCC.(21) About 340,000 liver cancer are caused by HBV virus.(17)

HBV is caused by a double-strand circular DNA molecule belonging to the family hepadnaviridae with 8 genotypes (A to H). According to appropriate data, genotypes A and B are predominantly found in Europe and the Middle East while B and C are chiefly in Asia. In many studies, genotype C has been considered as the major cause of HCC. The transmission of HBV through contaminated blood transfusion, intravenous injection and sexual contact and through the transmission mother to foetus (24) Confection with HBV is found in 9% of HIV infected patients.(25)

The mechanism of HCC due to HBV infection is unknown but it represents its pathogenic function through direct and indirect pathways. After the integration of viral DNA into hepatocytes, chromosomes contribute to oncogenesis. Another means of HBV related HCC carcinogenesis is due to a type of HBx viral protein. its bind to various cellular protein and causes chromosomal instability and modulate various oncogenic pathways resulting in cell proliferation, migration and viability. HBx also acts on p53 and deregulates the p53 mediated apoptosis. Secondly, HBV-related HCC may occur indirectly, inflammation and regeneration associated with chronic HBV infection via cirrhosis. (26-28)(29)

HCV infection

Hepatitis c virus (HCV) is a hepatotropic RNA virus belonging Flaviviridae family.(8) Studies of HCV infection so far have shown that it is found in variation in six different genotypes that show high genetic variability. Type I, II and III genotypes mainly found in western countries and type IV predominate in the Middle East. It is one of the leading causes of chronic liver disease. Once infected with HCV, 80% of patients found chronic hepatitis in developing countries whereas ~20% developing cirrhosis. Excessive alcohol intake in HCV infection increases the rate of HCC from 1.7 to 2.9.(24) HCV virus is not integrated into the host genome. HCV core protein possesses drive lipogenesis and influenced oxidative stress and inhibits the retinoblastoma protein and the p53 tumor suppressor gene leads to synergistic inhibition which causes greater degree of carcinogenesis. HCV Non-structural protein of HCV, NS3, and NS5A(17) are also responsible and mediators to induce fibrosis.(8, 17, 30, 31)

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a common liver disorder due to excessive accumulation of fats inside the liver which is responsible for chronic liver diseases in the USA.(32) The number of HCC from NAFLD has steadily increased at around 9% year. (33) It is the spectrum of diseases that causes numerous problems in the liver such as nonalcoholic steatohepatitis (NASH), steatosis, cirrhosis and hepatocellular carcinoma (HCC). The reason behind NAFLD is improper nutrition diet, obesity, insulin resistance, and metabolic syndrome.(34) Chronically moderate use of alcohol speed up the development of HCC. According to the current data, 0.5% steatosis and 2.8% NASH responsible for HCC. Various data of epidemiologic study shown that persons with metabolic syndrome such as diabetes mellitus with obesity are more vulnerable to NAFLD. Obesity also linked to the activation of inflammatory pathways of adipose tissues. (33)

Iron overload

Iron is an essential element for the body, but when its volume increases above normal, it causes unnecessary effects on the body. Maximum iron is usually present in the body as haemoglobin and in the liver. The intracellular protein ferritin inside the liver binds to 4500 molecules of elemental iron in the body. The problem of iron overload is mainly due to hereditary hemochromatosis (HH) and excessive dietary iron.(21, 35) Too much build-up of iron causes necrosis and apoptosis of hepatocytes via oxidative stress and damaging DNA, lipids and protein.(6) HH causes cirrhosis problems also that further promote HCC. Hepcidin is a major regulator of iron in the body. Low secretion of hepcidin associated with HH, which is caused by a mutation in the HFE gene.(35) It has been proved in animal studies that HCC develops with a high iron diet without cirrhosis or fibrosis.(36, 37)

Autoimmune hepatitis (AIH)

Autoimmune hepatitis (AIH) is a chronic liver disease in which immune system attacks on liver cells resulting in cirrhosis or fibrosis.(17, 38) Probable enhancer for AIH include the hepatitis B virus, cytomegalovirus, and the herpes simplex virus.(39) A recent study showed the HCC occurs rarely in patients with AIH (<1%) than for patients with cirrhosis from viral hepatitis or primary biliary cholangitis.(17, 40) Elevated levels of serological parameters such as aminotransferase and auto antibodies show that positive patient of AIH and differentiate between two types of diseases.(41)

Others chemical carcinogen

Various types of chemicals such as aflatoxin B_1 (AFB_1), vinyl chloride and alcohol are responsible to initiate HCC. Aflatoxin is a toxic metabolite formed by aspergillus flavus and aspergillus parasites originate in tropical and subtropical regions of the globe. Among various aflatoxin, AFB1 is more toxic and mutagenic to liver cells. Aflatoxin is carcinogenic in many animal species and co-occur *in-vivo* and *in-vitro* bind the guanine and cytosine residues of DNA and form AFB1-DNA adducts which ultimately affect the protein synthesis. It also produces mutation in tumour suppressor gene p53 at 249 codons. Vinyl chloride (VC) a wide-spread environmental contaminant leads to carcinogenesis in animals and humans. VC is a colourless toxic gas, it's activated by enzyme cytochrome p450 (CYP2E1) primarily in hepatocytes. After metabolic activation of VC may cause specific mutation in the liver.(42) Alcohol is placed in the 1st group chemical carcinogen category by the international agency. It also increases the risk of HCC by 3 to 10 fold in alcoholics.(43)

Role of biomarkers

Biomarker is a tool for diagnose, predict and prevent disease progression. The biomarkers can be measured in biochemical and serological parameters such as blood, urine, serum, and plasma which is very important in the early stage of cancer research.(44) Biomarkers are also capable of diagnosis particular types of disease as well as metastasis in distant organ.(45)

3. MOLECULAR AND BIOCHEMICAL CELLULAR MARKERS

Glypican-3 (GPC3)

GPC3, a member of the heparan sulfate (HS) proteoglycan family, attached to the cell membrane along with is often elevated in hepatocellular carcinoma (HCC) cases(46) but not detected in healthy adult liver.(47). GPC3 is located on chromosome xq26 and encodes GPC3. GPC3 possesses the main role in wnt and hedgehog cell signalling pathways, insulin-like growth factor, bone morphogenetic protein (BMP), fibroblast growth factor (FGF).(48) GPC3 released by the lipase Notum, therefore, it can be detected in serum(49) and possesses important roles in cell proliferation and tumour suppression, regulation in HCC tissues, while it is downregulated in lung adenocarcinoma, ovarian cancer, and breast cancer. Due to its ability to distinguish between well differentiated small tumour patients and those with cirrhosis. The terminal soluble fraction of GPC3 was proposed as a complimentary serologic biomarker.(50, 51)

A-fetoprotein

Alpha-fetoprotein (AFP) is a 70-kDa glycoprotein tumor marker elevated level found in HCC and gastrointestinal tumours. AFP is normally produced during foetal development reaches the maximum concentration (3g/L) during 12-16 weeks and exponentially declines after birth. AFP is found in three glycoforms such as AFP-L1, AFP-L2, and AFP-L3. Increased level of AFP- L1 found in cirrhosis and chronic hepatitis while AFP- L3 associated with HCC. AFP-L3 is generated by malignant cells consequently, it is regarded as a specific biomarker for HCC.(52-54)

Des-γ-carboxyprothrombin (DCP)

Des-γ-carboxyprothrombin (DCP) is a prothrombin precursor chiefly generated in hepatocellular carcinoma (HCC). Primarily it occurs in the absence of vitamin K. At least 44-81% of HCC patients found with an augmented level of DCP. Firstly Liebman et al.1984 reported a prominent serum DCP in HCC patient, by combining DCP with other specific marker give significant result in diagnosing of HCC patient.(55, 56) Two imperative reason behind DCP elevation in HCC is hypoxia and phenotypic changes in HCC cells in both the cases impairment of vitamin K were observed. Growth and metastasis of tumour cells in HCC through activation of various oncogenic pathways such as JAK-STAT, matrix metalloproteinase (MMPs) and ERK1/2 MAPK signalling pathway.(10, 54, 57)

Golgi glycoprotein – 73

Golgi protein 73 is a 73 kDa, Golgi transmembrane protein also known as Golph2. Its activate cAMP mediate signalling which enhanced transcription, matrix metalloproteinase (MMPs) and level of GP73. Fouad et al 2016 reported that GP73 generally expressed in biliary epithelial cells of liver but its expression is increased in certain disease conditions and found that GP73 has better sensitivity and specificity than AFP. However, there have been some controversial data that have denied the property of GP73.(57, 58)(59)

Osteopontin (OPN)

Osteopontin (OPN) is a type of glycoprotein which involved in several tumorigenic process. OPN expression elevated in so many cancers such as liver, gastric, rectal and ovarian cancer and also it promotes tumour metastasis and reoccurrence via PI3K/AKT signalling

pathway.(60, 61) In normal physiological conditions, OPN is not expressed in hepatocytes but found in some liver cells such as kupffer and stellate cells. Osteopontin (OPN) is an early-stage HCC biomarker with greater performance than AFP.(57, 62)

Hepatocyte paraffin 1 (Hep Par 1)

Hep Par 1 is an immunohistochemical marker found in mitochondria of liver cells and nowhere else. It is used to differentiate HCC from secondary metastatic liver cancer. A high expression of Hep Par 1 was found in the majority of HCC tumours (35 of 48) while showed low expression in tumours, such as the lung, gallbladder, stomach, pancreas, colon, and malignant melanoma.(44, 63, 64)

Squamous Cell Carcinoma Antigen (SCCA)

Squamous cell carcinoma antigen (SCCA) truly consists of two relatively homologous proteins (SCCAl and SCCA2) in the serine protease inhibitor family. Pontisso et al. 2004 and Cannito et al. 2014 studied the SCCA that was over expressed in tumour tissue of HCC patients and act as an apoptotic agent. This demonstrates that it is a potential histological marker for HCC diagnosis.(65, 66)

Heat shock protein 70 (HSP70)

Heat shock proteins (HSPs) are notably conserved proteins, HSPs play imperative roles in protein homeostasis, apoptosis, invasion, and cell signalling transduction. HSP are expressed at low ranges under normal conditions, however significantly triggered in response to cellular stresses, by heat shock factor-1 (HSF-1). In tumour invasion and metastasis, HSF-1 and HSP70 are chiefly involved.(67, 68)

Ki-67 antigen

Ki-67 is a form of nuclear protein, primarily present in the dense fibrillar components of nucleoli and cortices, which is associated with cellular proliferative activity.(69) higher expression of Ki-67 levels extensively correlating with greater degree of increases tumor grades I to IV in HCC and extend the possibilities of metastasis.(67)

BAX and BCL-2

Apoptosis-mediating factors characterized by a group of bcl-2 family, which include bcl-2, mcl-1, bcl-x, bax, bak, and many others. they contain apoptosis-promoting (bax, bak, bcl-xS) or apoptosis-inhibiting (bcl-2, mcl-1, bcl-xL) properties and evaluate for frequency of expression of apoptotic markers in hepatocellular carcinoma (HCC). Bax is shown in a kind of epithelial ovarian carcinomas, gastric carcinoma, gliomas, neuroblastomas, and medulloblastomas. Bax is expressed in a class of epithelial ovarian carcinomas, gastric carcinoma.(1)

Apolipoprotein J (Apo J)

Apo J additionally recognized as clusterin, have 75 - 80 kDa disulfide-linked heterodimeric protein with seven exclusive Glycosylated sites. Apo J is found in small amounts in HCC and normal in healthy control. Apo J also responsible for the development and metastasis of HCC. It can be used as a prognostic biomarker. To know more about Apo J contribution to HCC, further study is required.(67, 70)

Cancer stem cells marker (CSCs)

Hepatocellular carcinomas consist of a assorted group of cells that have varying capability to proliferate and seed new tumours, a subpopulation of HCC cells variously referred to as tumour initiating cells or Cancer Stem Cells (CSCs).(71) Feasible source of most cancers stem cells in the case of HCC is mutated hepatocyte.(72) CSCs quite resistant to chemotherapy, radiotherapy and exhibit remarkable efflux potential, dysregulation of signalling pathways, gradual growth and capacity to form spherical colonies. (72)

1. CD133

CD-133 is 97 kDa, referred to as prominin -1, is a type of transmembrane glycoprotein which is encoded by the PROM1 gene. Mostly it is expressed in the nucleus and cytoplasm of HCC tumours. CD133-positive cells confirmed a huge up regulation of Wnt /β -catenin, Notch, Hedgehog / SMO, Bmi, and Oct3/4 expression compared to the negative and show traditional CSC-like capabilities such as spheroid formation, chemoresistance, migration, and tumorigenesis.(73, 74)

2. CD13

CD13 known an aminopeptidase N is a membranous glycoprotein, which is linked with tumour progression and form clusters foci and is predominated in the G0 phase of the cell cycle. The excessive expression of CD13 causes early recurrence, terrible prognosis and shorter survival. The inhibition of CD13 blocks the tumour-initiating and self-renewal potential of CSCs.(75) Proliferation outcomes have in

addition established that CD13+ HCC cells are resistant to chemotherapy. CD13 can also minimize reactive oxygen species-induced DNA injury after chemotherapy or radiotherapy to stop HCC cells from apoptosis.(76)

CD44

CD44 is a multi-structural transmembrane glycoprotein, which participates in a couple of cellular processes which include cell growth, survival, differentiation and motility through works as a receptor for hyaluronic acid (HA) and as a co-receptor for growth factor and cytokines. CD44 is preferentially expressed in CD133+ populations and contributes to the CSC-like features synergistically. Thus, CD44 is typically used to distinguish CSC subpopulations in combination with different surface markers.(77)

EpCAM

Epithelial cell adhesion molecule (EpCAM) is a type I transmembrane glycoprotein which is often expressed in a couple of carcinomas. participates in cell migration, metastasis and works directly as a transcription factor that activates c-myc, cyclin A, and cyclin E in promoting cell cycles and proliferation.(73, 78) Yamashita et al. 2007 were one of the first to represent EpCAM in the HCC cell line, demonstrating that EpCAM-expressing cells have self-renewing and differentiating properties such as stem cells. The EpCAM antigen could be used to detect circulating tumor cells. It has been established that EpCAM positive cells play a relevant role in cancer development and have been recognized as a molecular biomarker for chemoresistance.(79)

MicroRNAs

miRNAs are quite short, single-stranded noncoding RNA molecules whose length from 19 to 25 nucleotides. At least 30% post transcriptional process controlled by miRNAs. Various miRNAs up or down regulate in specific cell or tissue according to disease condition.(57, 80) miRNAs regulate the progression of HCC and play a beneficial role in principal cellular pathways. MiRNAs play a vital role in cell development, proliferation, and apoptosis and regulate the expression of the main tumour-related genes in carcinogenesis, performing as oncogenes or tumour suppressor genes. Imbalance between miRNAs levels show specific disease condition and can be easily detected in various samples such as blood, serum, plasma, urine and faeces.(81).

Funding:

This study has not received any external funding.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

Peer-review:

External peer-review was done through double-blind method.

Data and materials availability:

All data associated with this study are present in the paper.

REFERENCE

1. Acharya SKJJoc, hepatology e. Epidemiology of hepatocellular carcinoma in India. 2014;4:S27-S33.
2. Ocker MJWjog. Biomarkers for hepatocellular carcinoma: What's new on the horizon? 2018;24(35):3974.
3. Kanwal F, Singal AGJG. Surveillance for hepatocellular carcinoma: Current best practice and future direction. 2019.
4. Ba M-C, Long H, Tang Y-Q, Cui S-ZJIjoc, pathology e. GP73 expression and its significance in the diagnosis of hepatocellular carcinoma: a review. 2012;5(9):874.
5. Aravalli RN, Steer CJ, Cressman EN. Molecular mechanisms of hepatocellular carcinoma. Hepatology. 2008;48(6):2047-63.
6. Röcken C, Carl-McGrath SJDD. Pathology and pathogenesis of hepatocellular carcinoma. 2001;19(4):269-78.
7. Whittaker S, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. Oncogene. 2010;29(36):4989-5005.
8. Axley P, Ahmed Z, Ravi S, Singal AKJJoc, hepatology t. Hepatitis C virus and hepatocellular carcinoma: a narrative review. 2018;6(1):79.
9. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi SJTLGH. Global burden of cancers attributable to infections in 2012: a synthetic analysis. 2016;4(9):e609-e16.
10. Lou J, Zhang L, Lv S, Zhang C, Jiang S. Biomarkers for Hepatocellular Carcinoma. Biomark Cancer. 2017;9:1-9.
11. Goossens N, Nakagawa S, Sun X, Hoshida YJTcr. Cancer biomarker discovery and validation. 2015;4(3):256.

12. Li L, Zhao H, Chen B, Huang K, Hao Z, Fan Z, et al. Noninvasive Identification of Immune-Related Biomarkers in Hepatocellular Carcinoma. J Oncol. 2019;2019:2531932.
13. Meguro M, Mizuguchi T, Kawamoto M, Hirata K. The molecular pathogenesis and clinical implications of hepatocellular carcinoma. Int J Hepatol. 2011;2011:818672.
14. De Stefano F, Chacon E, Turcios L, Marti F, Gedaly R. Novel biomarkers in hepatocellular carcinoma. Dig Liver Dis. 2018;50(11):1115-23.
15. Waller LP, Deshpande V, Pyrsopoulos N. Hepatocellular carcinoma: A comprehensive review. World J Hepatol. 2015;7(26):2648-63.
16. Tapper EB, Parikh NDJb. Mortality due to cirrhosis and liver cancer in the United States, 1999-2016: observational study. 2018;362:k2817.
17. Sanyal AJ, Yoon SK, Lencioni R. The etiology of hepatocellular carcinoma and consequences for treatment. Oncologist. 2010;15 Suppl 4:14-22.
18. Tilg H. Cytokines and liver diseases. Canadian journal of gastroenterology = Journal canadien de gastroenterologie. 2001;15(10):661-8.
19. Gurtsevitch VJB. Human oncogenic viruses: hepatitis B and hepatitis C viruses and their role in hepatocarcinogenesis. 2008;73(5):504-13.
20. Suh JK, Lee J, Lee J-H, Shin S, jin Tchoe H, Kwon J-WJPo. Risk factors for developing liver cancer in people with and without liver disease. 2018;13(10):e0206374.
21. Teodor Streba C, Constantin Vere C, Rogoveanu I, Dan Florescu N. Introductory Chapter: Etiology and Pathogenesis of Hepatocellular Carcinoma. Hepatocellular Carcinoma - Advances in Diagnosis and Treatment 2018.
22. Wiemann SU, Satyanarayana A, Tsahuridu M, Tillmann HL, Zender L, Klempnauer J, et al. Hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis. 2002;16(9):935-42.
23. Farazi PA, Glickman J, Horner J, DePinho RAJCr. Cooperative interactions of p53 mutation, telomere dysfunction, and chronic liver damage in hepatocellular carcinoma progression. 2006;66(9):4766-73.
24. Balogh J, Victor D, 3rd, Asham EH, Burroughs SG, Boktour M, Saharia A, et al. Hepatocellular carcinoma: a review. J Hepatocell Carcinoma. 2016;3:41-53.
25. Konopnicki D, Mocroft A, De Wit S, Antunes F, Ledergerber B, Katlama C, et al. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. 2005;19(6):593-601.
26. Kew M, Hodkinson J, Paterson A, Song EJJomv. Hepatitis-B virus infection in black children with hepatocellular carcinoma. 1982;9(3):201-7.
27. Kew MC, Miller RH, Chen H-S, Tennant BC, Purcell RHJPotNAoS. Mutant woodchuck hepatitis virus genomes from virions resemble rearranged hepadnaviral integrants in hepatocellular carcinoma. 1993;90(21):10211-5.
28. Yang H-I, Lu S-N, Liaw Y-F, You S-L, Sun C-A, Wang L-Y, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. 2002;347(3):168-74.
29. Xie Y. Hepatitis B Virus-Associated Hepatocellular Carcinoma. Advances in experimental medicine and biology. 2017;1018:11-21.
30. Nault JC, Calderaro J, Di Tommaso L, Balabaud C, Zafrani ES, Bioulac-Sage P, et al. Telomerase reverse transcriptase promoter mutation is an early somatic genetic alteration in the transformation of premalignant nodules in hepatocellular carcinoma on cirrhosis. 2014;60(6):1983-92.
31. Tomasetti C, Li L, Vogelstein BJS. Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. 2017;355(6331):1330-4.
32. Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. J Clin Gastroenterol. 2013;47 Suppl:S2-6.
33. Tiniakos DG, Maurício J, Reeves HL. Fatty Liver Disease and Hepatocellular Carcinoma: The Pathologist's View. Alcohol and Cancer: Springer; 2018. p. 55-69.
34. Zhang X. NAFLD Related-HCC: The Relationship with Metabolic Disorders. Adv Exp Med Biol. 2018;1061:55-62.
35. Kew MC. Hepatic iron overload and hepatocellular carcinoma. Liver cancer. 2014;3(1):31-40.
36. Pietrangelo AJNEJoM. Hereditary hemochromatosis—a new look at an old disease. 2004;350(23):2383-97.
37. Friedman B, Baynes R, Bothwell T, Gordeuk V, Macfarlane B, Lamparelli R, et al. Dietary iron overload in southern African rural blacks. 1990;78(9):301-5.
38. Park SZ, Nagorney DM, Czaja AJ. Hepatocellular carcinoma in autoimmune hepatitis. Dig Dis Sci. 2000;45(10):1944-8.
39. Bogdanos DP, Choudhuri K, Vergani DJL. Molecular mimicry and autoimmune liver disease: virtuous intentions, malign consequences. 2001;21(4):225-32.
40. Wong RJ, Gish R, Frederick T, Bzowej N, Frenette C. Development of hepatocellular carcinoma in autoimmune hepatitis patients: a case series. Dig Dis Sci. 2011;56(2):578-85.
41. Vergani D, Alvarez F, Bianchi FB, Cançado EL, Mackay IR, Manns MP, et al. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. 2004;41(4):677-83.
42. Zhang Y-JJWjoh. Interactions of chemical carcinogens and genetic variation in hepatocellular carcinoma. 2010;2(3):94.
43. Matsushita H, Takaki AJBog. Alcohol and hepatocellular carcinoma. 2019;6(1):e000260.

44. Chauhan R, Lahiri N. Tissue- and Serum-Associated Biomarkers of Hepatocellular Carcinoma. Biomark Cancer. 2016;8(Suppl 1):37-55.
45. Leong PP, Koch WM, Reed A, Eisele D, Sidransky D, Jen J, et al. Distinguishing second primary tumors from lung metastases in patients with head and neck sauamous cell carcinoma. 1998;90(13):972-7.
46. Wu Y, Liu H, Ding HJJohc. GPC-3 in hepatocellular carcinoma: current perspectives. 2016;3:63.
47. Zhou F, Shang W, Yu X, Tian JJMrr. Glypican-3: A promising biomarker for hepatocellular carcinoma diagnosis and treatment. 2018;38(2):741-67.
48. Zhang L, Liu H, Sun L, Li N, Ding H, Zheng J. Glypican-3 as a potential differential diagnosis marker for hepatocellular carcinoma: a tissue microarray-based study. Acta Histochem. 2012;114(6):547-52.
49. Filmus J, Capurro MJCC. The role of glypican-3 in the regulation of body size and cancer. 2008;7(18):2787-90.
50. Hippo Y, Watanabe K, Watanabe A, Midorikawa Y, Yamamoto S, Ihara S, et al. Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. 2004;64(7):2418-23.
51. Sung YK, Hwang SY, Park MK, Farooq M, Han IS, Bae HI, et al. Glypican-3 is overexpressed in human hepatocellular carcinoma. 2003;94(3):259-62.
52. Chen D-S, Sung J-LJNEJoM. Hepatitis B virus infection on Taiwan. 1977;297(12):668-9.
53. Murugavel KG, Mathews S, Jayanthi V, Shankar EM, Hari R, Surendran R, et al. Alpha-fetoprotein as a tumor marker in hepatocellular carcinoma: investigations in south Indian subjects with hepatotropic virus and aflatoxin etiologies. 2008;12(6):e71-e6.
54. Singh AK, Kumar R, Pandey AK. Hepatocellular Carcinoma: Causes, Mechanism of Progression and Biomarkers. Curr Chem Genom Transl Med. 2018;12:9-26.
55. Ette AI, Ndububa DA, Adekanle O, Ekrikpo U. Utility of serum des-gamma-carboxyprothrombin in the diagnosis of hepatocellular carcinoma among Nigerians, a case–control study. BMC Gastroenterol. 2015;15(1):113.
56. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo K-J, Lee S-D, et al. Des-γ-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. 1984;310(22):1427-31.
57. De Stefano F, Chacon E, Turcios L, Marti F, Gedaly RJD, Disease L. Novel biomarkers in hepatocellular carcinoma. 2018;50(11):1115-23.
58. Ai N, Liu W, Li Z-G, Ji H, Li B, Yang G. High expression of GP73 in primary hepatocellular carcinoma and its function in the assessment of transcatheter arterial chemoembolization. Oncol Lett. 2017;14(4):3953-8.
59. Fouad M, Haleim E, Azeim W, Elela D, Abdelsalam O. Golgi protein 73 versus alpha fetoprotein as a marker for hepatocellular carcinoma. 2016;29(1):152-9.
60. Cabiati M, Gaggini M, Cesare MM, Caselli C, De Simone P, Filipponi F, et al. Osteopontin in hepatocellular carcinoma: A possible biomarker for diagnosis and follow-up. 2017;99:59-65.
61. Duarte-Salles T, Misra S, Stepien M, Plymoth A, Muller D, Overvad K, et al. Circulating osteopontin and prediction of hepatocellular carcinoma development in a large European population. 2016;9(9):758-65.
62. Shang S, Plymoth A, Ge S, Feng Z, Rosen HR, Sangrajrang S, et al. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. 2012;55(2):483-90.
63. Fasano M, Theise ND, Nalesnik M, Goswami S, Garcia de Davila MT, Finegold MJ, et al. Immunohistochemical evaluation of hepatoblastomas with use of the hepatocyte-specific marker, hepatocyte paraffin 1, and the polyclonal anti-carcinoembryonic antigen. Mod Pathol. 1998;11(10):934-8.
64. Fan Z, van de Rijn M, Montgomery K, Rouse RV. Hep par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumors tested using tissue microarrays and conventional tissue sections. Mod Pathol. 2003;16(2):137-44.
65. Yu J, Wang Z-J, Chen L-H, Dong W-ZJSjoc, investigation l. Diagnostic value of serum squamous cell carcinoma antigen for hepatocellular carcinoma: a systematic review and meta-analysis. 2017;77(1):8-14.
66. Cannito S, Turato C, Paternostro C, Biasiolo A, Colombatto S, Cambieri I, et al. Hypoxia up-regulates SERPINB3 through HIF-2α in human liver cancer cells. 2015;6(4):2206.
67. Chauhan R, Lahiri NJBic. Tissue-and serum-associated biomarkers of hepatocellular carcinoma. 2016;8:BIC. S34413.
68. Wang C, Zhang Y, Guo K, Wang N, Jin H, Liu Y, et al. Heat shock proteins in hepatocellular carcinoma: Molecular mechanism and therapeutic potential. 2016;138(8):1824-34.
69. He Y, Liu F, Mou S, Li Q, Wang SJOl. Prognostic analysis of hepatocellular carcinoma on the background of liver cirrhosis via contrast-enhanced ultrasound and pathology. 2018;15(3):3746-52.
70. Comunale MA, Wang M, Rodemich-Betesh L, Hafner J, Lamontagne A, Klein A, et al. Novel changes in glycosylation of serum Apo-J in patients with hepatocellular carcinoma. 2011;20(6):1222-9.
71. Mansour AH, Elkhadry T, Anwar R, Habeeb MR, Mohammed MAJIJCR. Regulation of Cancer Stem Cell Marker (CD133) by transforming growth factor Beta in hepatocellular carcinoma. 2014;10:65-73.
72. Wang K, Sun D. Cancer stem cells of hepatocellular carcinoma. Oncotarget. 2018;9(33):23306-14.

73. Wang N, Wang S, Li M-Y, Hu B-G, Liu L-P, Yang S-L, et al. Cancer stem cells in hepatocellular carcinoma: an overview and promising therapeutic strategies. Ther Adv Med Oncol. 2018;10:1758835918816287-.
74. Yin S, Li J, Hu C, Chen X, Yao M, Yan M, et al. CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. 2007;120(7):1444-50.
75. Haraguchi N, Ishii H, Mimori K, Tanaka F, Ohkuma M, Kim HM, et al. CD13 is a therapeutic target in human liver cancer stem cells. 2010;120(9):3326-39.
76. Wang N, Wang S, Li M-Y, Hu B-g, Liu L-p, Yang S-l, et al. Cancer stem cells in hepatocellular carcinoma: an overview and promising therapeutic strategies. 2018;10:1758835918816287.
77. Yan Y, Zuo X, Wei DJSctm. Concise review: emerging role of CD44 in cancer stem cells: a promising biomarker and therapeutic target. 2015;4(9):1033-43.
78. Lima LdP, Machado CJ, Rodrigues JBSR, Vasconcellos LdS, Junior EP, Vidigal PVT, et al. Immunohistochemical Coexpression of Epithelial Cell Adhesion Molecule and Alpha-Fetoprotein in Hepatocellular Carcinoma. 2018;2018.
79. Yamashita T, Budhu A, Forgues M, Wang XWJCr. Activation of hepatic stem cell marker EpCAM by Wnt–β-catenin signaling in hepatocellular carcinoma. 2007;67(22):10831-9.
80. Gong J, He X-X, Tian D-AJOI. Emerging role of microRNA in hepatocellular carcinoma. 2015;9(3):1027-33.
81. Xu X, Tao Y, Shan L, Chen R, Jiang H, Qian Z, et al. The Role of MicroRNAs in Hepatocellular Carcinoma. J Cancer. 2018;9(19):3557-69.